Effects of Developmental Exposure to Polychlorinated Biphenyls and/or Polybrominated Diphenyl Ethers on Cochlear Function

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Developmental exposure to polychlorinated biphenyls (PCBs) causes hearing loss that may be due to reduced thyroxine during cochlear development. Polychlorinated diphenyl ethers (PCDDs) are structurally similar to PCBs and reduce thyroxine. This study utilized an environmental PCB mixture and a commercial PBDE mixture, DE-71, that represents the PBDEs found in humans to assess the potential for additive effects of PCBs and PBDEs on cochlear function. Female Long-Evans rats were dosed with corn oil vehicle, PCBs (3 or 6 mg/kg), molar equivalent doses of PBDEs (5.7 or 11.4 mg/kg), 3 mg/kg PCBs + 5.7 mg/kg PBDEs, or 6 mg/kg PCBs + 11.4 mg/kg PBDEs throughout gestation and lactation. At weaning, pup blood was taken to assess thyroxine concentrations. One male and one female from each litter were maintained until adulthood for distortion product otoacoustic emission (DPOAE) measurements of cochlear function. DPOAE amplitudes were decreased and thresholds were elevated in the 6 mg/kg PCB group. Exposure to PBDEs did not cause DPOAE deficits. There was an interactive effect from combined exposure such that the individual low doses of PCBs and PBDEs did not result in DPOAE deficits, but the two combined produced a deficit similar to that in the high-dose PCB group. Serum thyroxine concentrations of all groups were reduced compared with controls, but PBDEs produced a less dramatic reduction than PCBs, which could explain the lack of DPOAE effects. Importantly, there was evidence that the co-exposure to subthreshold doses of PCBs and PBDEs can have an additive effect on cochlear function.

Key Words: PCBs; PBDEs; auditory system; DPOAE.
reported to elevate brain auditory evoked potential thresholds, as well as prolong the latencies of waves II and IV (Lilienthal et al., 2011). The authors speculated that because the latencies of waves II and IV were prolonged to the same degree relative to waves II and IV in controls, this indicates a cochlear site of action rather than along the level of the ascending auditory pathway.

There are limited human studies assessing the impact of PCB exposure on auditory function, but several studies suggest that prenatal exposure may lead to subtle auditory deficits. A study in the Faroe Islands found that higher prenatal PCB exposure was associated with increased auditory thresholds at 250 and 12,000 Hz but only in the left ear (Grandjean et al., 2001). In another study, higher PCB concentrations in maternal serum were associated with slightly increased hearing thresholds but only at 2000 Hz in the left ear and 4000 Hz in the right ear (Longnecker et al., 2004). More recently, hearing of a cohort of 8- to 9-year-old Slovakian children with higher serum PCB concentrations than the children assessed previously was measured using transient otoacoustic emissions (TEOAEs), a technique that specifically assessed the integrity of the outer hair cells in the cochlea. In these children, higher serum PCB concentrations were associated with low-frequency hearing loss as indicated by reduced TEOAE amplitudes and increased TEOAE thresholds (Trnovec et al., 2008).

These same children were tested again at age 12 using both TEOAEs and DPOAEs and an association between higher PCB concentrations and reduced TEOAE and DPOAE amplitudes was observed. Again, this effect was observed primarily at low frequencies (Trnovec et al., 2010).

Exposure to PBDEs has been shown to reduce circulating thyroid hormone concentrations in humans and rodents (Turky et al., 2008; Zhou et al., 2002). However, to date, there are no published human or animal studies assessing the impact of developmental PBDE exposure on cochlear function. The present study used a rat model to investigate developmental exposure to PBDEs either alone or in combination with PCBs as a possible cause of cochlear dysfunction. PBDE doses were the molar equivalents of PCB doses previously demonstrated to produce DPOAE deficits (Powers et al., 2006). The goal was to determine if developmental exposure to PBDEs could cause cochlear dysfunction and if the combination of the two compounds would have an interactive effect on outer hair cell integrity.

**MATERIALS AND METHODS**

**Animals.** One hundred and seven primiparous female Long-Evans rats, approximately 60 days of age, were purchased from Harlan (Indianapolis, IN) in three cohorts spaced approximately 6 months apart. The animals were maintained in facilities accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care. Specifically, rats were individually housed in standard polycarbonate plastic shoebox cages with corn cob bedding, in a temperature- and humidity-controlled room (22°C, 40–55% humidity) on a 12/12-h reverse light/dark cycle (lights off at 0830 h). Food and water were available ad libitum. Rats were fed Harlan Teklad rodent diet (W) 8604. All procedures were approved by the Institutional Animal Care and Use Committee at the University of Illinois at Urbana-Champaign and were in accordance with the guidelines of the National Institutes of Health (2002) and National Research Council Institute for Laboratory Animal Research (2003).

**Exposure.** After 1 week of adaptation to the vivarium, the rats in each cohort were assigned to exposure groups (distributed evenly by body weight) and given one of the seven treatments consisting of PCBs and/or PBDEs in corn oil or corn oil vehicle only. Each exposure group was represented in each cohort. Exposure began 28 days before breeding and continued until pups were weaned on postnatal day (PND) 21. The PCB mixture (Fox River PCB mixture) was formulated to mimic the congener profile found in walleye, a popular sport-caught fish, taken from the Fox River in northeast Wisconsin. The mixture consisted of 35% Aroclor 1242 (Monsanto lot KB 05-415, St Louis, MO) 35% Aroclor 1248 (AccuStandards lot F-110, New Haven, CT), 15% Aroclor 1254 (Monsanto lot KB 05-612), and 15% Aroclor 1260 (AccuStandards lot 021-020) (Kostyniak et al., 2005). The doses of the PCB mixture (3 and 6 mg/kg/day) were selected based on the results of earlier studies assessing the in vivo developmental toxicity and auditory toxicity of the mixture in rats (see Kostyniak et al., 2005; Powers et al., 2006). The PBDE mixture was DE-71 (Cambridge Isotope Lab lot 11-9570, Andover, MA), a discontinued technical commercial PBDE mixture mainly consisting of 42–44% BDE-99 and 36% BDE-47, the two PBDEs that are most prevalent in human tissue. The PBDE doses were 5.7 and 11.4 mg/kg/day, the molar equivalents to the 3 and 6 mg/kg/day PCB doses. The low combined dose was 3 mg/kg/day PCB plus 5.7 mg/kg/day PBDE, and the high combined dose was 6 mg/kg/day PCB plus 11.4 mg/kg/day PBDE. The PCB and PBDE mixtures were diluted in corn oil (Mazola) and pipetted onto one half of a vanilla wafer cookie at a volume of 0.4 ml/kg body weight of the rat (Keebler Golden Wafers). To arrive at a volume of 0.4 ml/kg body weight, the Fox River mix and DE-71 were diluted in corn oil to achieve concentrations of 7.5 and 15 mg/ml for the PCB doses of 3 and 6 mg/kg and 14.25 and 28.5 mg/ml for the PBDE doses of 5.7 and 11.4 mg/kg, respectively. The PB- and/or PBDE-treated cookies were fed to the female rats daily at approximately 1100 h. The amount of dosing solution applied to the cookies was adjusted daily to account for weight gain. Corn oil vehicle alone was pipetted onto cookies for rats in the vehicle control group.

**Breeding, pregnancy, and weaning.** Four weeks after the initiation of exposure, each female was paired with an unexposed male Long-Evans rat (Harlan) in a hanging wire cage for eight consecutive days. The breeding cages contained standard rat chow and standard tap water (ad libitum). The females were returned to their home cages each day for PCB and/or PBDE dosing. Consumption of the cookie was confirmed before the females were returned to the breeding cages. The females were monitored twice daily for the presence of a sperm plug in order to establish gestational day 0. Females that did not give birth were retained and their uteri examined for implantation sites.

On the day of parturition (PND 0), the pups were examined for gross abnormalities, sexed and weighed, and the number of stillbirths was noted. On PND 2, the litters were reduced to 10 pups (five males and five females when possible), and extra pups were cross fostered to litters with at least 7 pups from the same treatment group to bring them to 8–10 pups. Cross-fostered pups were marked by ear clip and were not used for auditory testing. The day of eye opening was recorded. Pups were weighed weekly. There were 83 successful litters. Of the remaining females, 10 were not pregnant and 14 had litters that were too small (≤ 7 pups) to be included in this study. Overall, the nonpregnant females and small litters were relatively evenly distributed across treatment groups.

Dosing continued until the pups were weaned on PND 21. On PND 21, the dams were euthanized and the liver weight and number of uterine implantation sites were recorded. Two pups per litter, one male and one female, were retained for the auditory testing. Organ weights (brain, liver, and thymus) were obtained from one male and female per litter, and serum was collected for measurement of total thyroxine (T4) concentration. Pups retained on the day of weaning were housed in same exposure, same sex pairs, or triplets with food...
and water available ad libitum. Auditory testing began at approximately PND 100 to determine if developmental exposures to PCBs and/or PBDEs resulted in permanent hearing deficits.

**Total thyroxine radioimmunoassay.** Serum T4 concentration was measured by radioimmunoassay modified for rat serum as reported previously by Eltom et al. (1992) with further modifications to improve sensitivity according to the method of Schneider et al. (2006). The assay was conducted over 5 days. On the first day, 10 μl of serum was added to 200 μl of GAB buffer (0.2M glycine, 0.13M sodium acetate trihydrate, and 0.02% bovine serum albumin, pH 8.6) L-T4 standards equivalent to 10,000 for nonspecific binding or 620, 310, 155, 77.5, 38.8, 19.4, 9.7, 4.85, 2.43, 1.22, 0.61, and 0 pg were included to create the standard curve. Next, 100 μl of GAB containing 2 mg/ml of 8-anilino-1-naphthalene-sulfonic acid (ANS; Sigma) was added. The primary antibody used was a polyclonal rabbit anti-T4 antibody (Cat#20-TR40; Fitzgerald Industries International, Concord, MA). Approximately 0.006 μCi of [125I]-T4 was added on the third day. On the fifth day, 50 μl of a 200 μg/ml (10 μg) solution of rabbit immunoglobulin (Cat# 15006; Sigma) was added, followed by 100 μl of a GAR secondary antibody solution (Cat#R0881; Sigma) prepared at 60% of the manufacturers’ recommended volume for a final dilution of approximately 1:8. Tubes were incubated at room temperature for 30 min before addition of 1 ml of a 25% wt/vol solution of polyethylene glycol in PBS (0.01M NaCl and 0.01M NaH₂PO₄, pH 7.5). Tubes were then centrifuged, aspirated, and counted in a gamma counter (Packard Cobra Autogamma II). Data were linearized by log-logit regression. All samples in this study were run in a single assay. The serum T4 assay had a limit of detection of 1.0 nmol/l with 10 μl of serum or 0.8 μg per tube. The intraassay CV at 46.3 nmol/l (36 μg per tube) was 10.5%.

**Distortion product otoacoustic emissions.** DPOAEs are acoustic responses generated when the cochlea is stimulated by two pure tones (called f₁ and f₂ primaries). Loss of outer hair cells or impairment of outer hair cell function results in DPOAE deficits. DPOAE testing was conducted in a sound-attenuated chamber, lined with sound-absorbing foam, within a quiet isolated laboratory. Before testing, rats were sedated with 0.5 ml/kg ketamine/xylazine (87:13) ip. Once sedated, rats were placed on a thermo-regulating heating pad (no. 50-7053-R; Harvard Apparatus, Holliston, MA) to maintain body temperature. Rats were placed on their sides, and the DPOAE probe was positioned in the left ear canal.

DPOAEs were recorded using Tucker Davis Technologies (TDT, Alachua, FL) System 2/ System 3 digital signal processing hardware and software. Stimuli were directed into the ear canal through a single ear probe. The probe contained two earphones and a microphone and had a soft rubber tip that sealed the ear canal from external noise. All DPOAE stimuli were created using TDT SigGen software, and recordings were made using TDT BioSig software.

Details regarding the instrumentation can be found in Powers et al. (2006).

The DPOAEs were generated by simultaneously presenting two sinusoïds, f₁ and f₂ (f₂/f₁ = 1.2), into the sealed ear canal of the rat. The sound levels for the f₁ and f₂ primaries were calibrated to 60 dB sound pressure level (SPL) and 50 dB SPL (0 dB = 20 μPa), respectively, using a pressure-field microphone (no. 4192; Bruel and Kjaer, Norcross, GA) in a 2-cc calibration coupler (no. 4946; Bruel and Kjaer). The amplitude of the distortion product at the frequency defined by 2f₁ – f₂ was measured by recording the measured sound pressure in the sealed ear canal. In mammals, the 2f₁ – f₂ distortion product is the most robust and is commonly measured as a reliable indicator of outer hair cell function (Lonsbury-Martin and Martin, 1990). Six stimulus pairs were selected for DPOAE testing (f₂ = 2, 3, 4, 6, 8, and 12 kHz). Figure 1 shows an example of recorded DPOAE output of the f₁ and f₂ primaries as well as the 2f₁ – f₂ distortion product.

DPOAE testing consisted of measuring suprathreshold DPOAE amplitudes followed by DPOAE thresholds at each of the six stimulus pairs, beginning with 2 kHz. Each distortion product was the average response of 100 stimulus pair presentations (presentation rate = 6/s). The final distortion product represented an average of two trials (each having 100 separate stimulus presentations). DPOAE amplitudes were calculated by subtracting surrounding noise from the 2f₁ – f₂ distortion product. The noise was defined as the average of the 10 neighboring frequencies (five above and five below the 2f₁ – f₂ distortion product). After the suprathreshold amplitudes were measured, DPOAE thresholds were then determined by reducing the f₁ and f₂ primaries in 5-dB steps. Thresholds were defined as the lowest f₂ dB level at which the 2f₁ – f₂ distortion product was > 6 dB above the surrounding noise.

**Statistical analysis.** All statistical analyses were conducted using SPSS for MS Windows (version 15.0; SPSS Inc., Chicago, IL) with statistical significance set at p < 0.05. In some cases of repeated measures factors, a sphericity violation occurred. In such cases, a Greenhouse-Geisser correction was used to reduce the risk of a type I error if ε < 0.75 and a Huynh-Feldt correction was used when ε > 0.75. DPOAE amplitudes and DPOAE thresholds were analyzed via separate three-way ANOVAs with treatment as a between-subjects variable, frequency as a repeated measure, litter as the unit of variance, and sex nested within litter. Post hoc comparisons were conducted using Tukey’s test to examine the nature of significant treatment effects obtained from the overall analyses. Reproductive data included litter size, percent male births, percent live births, percent gestational weight gain, percent lactational weight gain, ratio of liver:body weight, and uterine implantation sites in the dam at weaning. For each dependent variable, ANOVA was conducted using treatment as a between-subjects factor. When significant treatment effects were obtained, post hoc Tukey’s tests were done to allow comparisons between treatment groups and the control group as well as comparisons by low- and high-dose treatment groups and between single and combined treatment groups.

Developmental data included average day of eye opening, postnatal weight gain, and organ:body weight ratios of the pups. Postnatal weight gain was determined by body weights on PND 0, 7, 14, and 21. These data were analyzed via mixed ANOVA with treatment as a between-subjects factor and sex (nested within litter) and age as repeated measures factor. Organ:body weight ratios for the brain, liver, and thymus were measured at the day of weaning and analyzed via mixed ANOVA with treatment as a between-subjects factor and litter as a unit of variance with sex nested within litter. Post hoc Tukey’s tests were conducted to examine treatment effects. Thyroid hormone data (T4) was analyzed via ANOVA with treatment as a between-subjects factor and sex nested within litter.

**RESULTS**

**Reproductive/Developmental EndPoints**

No clinical signs of overt toxicity were noted in the dams from any of the treatment groups, and all results seen here were...
similar to the findings in previous studies in our laboratory using similar doses of the Fox River PCB mixture. There were no significant treatment effects on percent gestational weight gain, percent lactational weight gain, dam liver weight to body weight ratio, litter size, percent male births, percent live births per litter, or implantation sites. There was a main effect of treatment \[ F(6,76) = 2.654, p = 0.022 \] on day of eye opening in pups. Tukey’s test revealed that the 6 mg/kg PCB group opened their eyes significantly earlier than the 5.7 mg/kg PBDE group \( (p = 0.04) \) and the 11.4 mg/kg PBDE group \( (p = 0.012) \), but none of the treatment groups differed significantly from the control group. There was no significant treatment \( \times \) sex interaction.

Analysis of pup postnatal weight gain revealed a main effect of treatment \[ F(6,76) = 13.218, p < 0.001 \] and a significant treatment \( \times \) time interaction \[ F(8,318.105,363) = 11.486, p < 0.001 \]. Simple ANOVA revealed a significant effect of treatment at each recorded time-point (PND 0, 7, 14, and 21). Significant differences at each time-point were driven primarily by lower body weights in the 6 mg/kg PCB and the high combined treatment groups compared with other treatment groups. Rats in these groups weighed about 18% less than those in the control group at weaning.

Analysis of brain:body weight ratio revealed a main effect of treatment \[ F(6,76) = 8.948, p < 0.001 \], and Tukey’s tests revealed that this difference was driven by higher brain:body weight ratios in the 6 mg/kg PCB and high combined groups. Analysis of liver:body weight ratio also revealed a main effect of treatment \[ F(6,76) = 114.151, p < 0.001 \], and Tukey’s test revealed that all treatment groups had significantly higher liver:body weight ratios than the control group. Finally, analysis of thymus:body weight ratio revealed a main effect of treatment \[ F(6,76) = 15.562, p < 0.001 \], and Tukey’s test revealed all exposure groups that received PCBs had significantly lower thymus:body weight ratios than controls.

**Distortion Product Otoacoustic Emission**

The numbers of litters used in the analysis of DPOAEs were 10, 10, 13, 11, 12, 11, and 10 for the control, 3 mg/kg PCB, 6 mg/kg PCB, 5.7 mg/kg PBDE, 11.4 mg/kg PBDE, low combined, and high combined groups. Figure 2 illustrates DPOAE amplitudes at \( f_2 \) frequencies of 2, 3, 4, 6, 8, and 12 kHz. There was a significant main effect of treatment \[ F(6,70) = 20.035, p < 0.001 \], a significant main effect of frequency \[ F(2.892,202.409) = 126.97, p < 0.001 \], and a sex \( \times \) frequency interaction \[ F(3.683,257.809) = 4.67, p = 0.002 \]. Comparisons of the sexes revealed that DPOAE amplitudes of male rats were lower than females at 12 kHz (graph not shown). There was no significant main effect of sex and no treatment \( \times \) sex interactions. Tukey’s tests revealed that DPOAE amplitudes were significantly lower in the 6 mg/kg PCB \( (p < 0.001) \), low combined \( (p = 0.001) \), and high combined \( (p < 0.001) \) groups compared with control group. Neither of the PBDE-alone groups differed significantly from the control group.

Figure 3 illustrates DPOAE thresholds at \( f_2 \) frequencies of 2, 3, 4, 6, 8, and 12 kHz. There was a significant main effect of treatment \[ F(6,70) = 15.342, p < 0.001 \] and a significant main effect of frequency \[ F(4.465,312.535) = 297.915, p < 0.001 \]. There was no significant main effect of sex and no sex \( \times \) treatment or frequency \( \times \) treatment interactions. Tukey’s tests revealed that DPOAE thresholds were significantly higher in the 6 mg/kg PCB \( (p < 0.001) \), low combined \( (p = 0.001) \), and high combined \( (p < 0.001) \) groups compared with control group. Both of the PBDE-alone groups did not differ significantly from the control group.

**Thyroxine Concentrations**

Figure 4 illustrates serum total T4 concentrations measured in littersmates of the rats that underwent DPOAE testing. T4
was measured at weaning (PND 21). There was a significant effect of treatment \( F(6,61) = 89.222, p < 0.001 \). Tukey’s tests revealed that all treatment groups had significantly lower T4 levels than the control group \( (p < 0.001) \), but the 5.7 mg/kg PBDE and the 11.4 mg/kg PBDE groups had significantly higher T4 levels than the PCB-alone and combined groups \( (p < 0.05) \).

**DISCUSSION**

This study confirmed that developmental exposure to the Fox River PCB mixture can result in deficits in cochlear function that last into adulthood. Decreased DPOAE amplitudes and increased thresholds were present across a range of frequencies, consistent with the results of previous studies in our laboratory \( \text{(Powers et al., 2006, 2009)} \). On the other hand, developmental exposure to the PBDE mixture, DE-71 at molar equivalent doses did not produce any DPOAE deficits. Interestingly, combined exposure to subthreshold doses of PCBs and PBDEs did produce significant hearing deficits, suggesting an additive effect.

Thyroid hormones are very important in the development of the cochlea, and hypothyroidism during the developmental period can lead to permanent hearing deficits \( \text{(Uziel, 1986)} \). It is well established that exposure to PCBs, especially during the postnatal or lactational period when rodent cochlear development is occurring, causes auditory deficits that persist into adulthood \( \text{(Crofton et al., 2000b)} \). Previous data suggest that a 60% decrease in T4 levels during the critical period for cochlear development is needed to result in hearing deficits \( \text{(Crofton, 2004; Crofton and Zoeller, 2005)} \). In this study, the Fox River PCB mixture was shown to reduce serum total T4 concentrations more than 60% at weaning compared with the control group, whereas molar equivalent doses of DE-71 produced a smaller effect on T4 and did not reduce the concentrations by more than 60%. Developmental exposure to PBDEs alone did not produce any DPOAE deficits. This is consistent with a previous study that exposed rodents to PBDEs \( \text{(Bromkal 70-5 DE or DE-47)} \) and isomolar equivalent PCB doses \( \text{(Aroclor 1254 or PCB 105)} \) and found that PCBs were more potent in reducing T4 concentrations than PBDEs \( \text{(Hallgren et al., 2001)} \).

Although reduced T4 concentrations may play a role in the cochlear dysfunction we observed, the sharp delineation between no effect in the DE-71 groups and clear cochlear dysfunction in the 6 mg/kg PCB or combined PCB/PBDE groups, with a relatively modest further decrease in T4 concentrations, suggests
that reductions in T4 may only be part of the story. In addition, T4 concentrations differed only slightly in the 3 and 6 mg/kg PCB-exposed groups, yet the 6 mg/kg dose group showed a clear deficit in cochlear function, whereas the 3 mg/kg group did not.

PCBs have been shown to bind to the ryanodine-receptor (RyR) and stabilize the high-affinity binding conformation of the receptor, thereby increasing intracellular calcium concentrations (Wong and Pessah, 1997). In particular, the Fox River Mix has been shown to have RyR activity (Kostyniak et al., 2005) and enhance the release of calcium from intracellular stores (Pessah et al., 2006). RyR has been identified in the rat cochlea and its localization includes the inner and outer hair cells (Beurg et al., 2005; Morton-Jones et al., 2006). An increase in intracellular calcium concentrations through activation of the RyR may regulate the motion of the outer hair cells (Dallos and Harris, 1978), and thus, increased RyR activation could potentially contribute to the observed cochlear deficits. Studies have demonstrated that administration of drugs (caffeine and ryanodine) that induce calcium release from RyR stores into the perilymph reversibly suppressed DPOAEs (Bobbin, 2002). Interestingly, developmental exposure to PCB-95, a potent activator of the RyR with only a modest effect on thyroxine concentrations, severely disrupted the tonotopic map of the auditory cortex but did not result in hearing loss as measured by the auditory brain stem response (ABR) (Kenet et al., 2007).

PCBs and PBDEs can also disrupt neurotransmitter systems and induce oxidative stress, and these effects could play a role in cochlear dysfunction. Noncoplanar PCBs can inhibit plasma membrane uptake of glutamate (Mariussen and Fonnum, 2001), which plays important roles in the cochlea, whereas the pentabDEs were generally a poor plasma membrane uptake inhibitor (Mariussen and Fonnum, 2003). More exogenous glutamate excitotoxicity during cochlear development can lead to elevated hearing thresholds in rats (Janssen et al., 1991). PCBs have also been shown to increase reactive oxygen species formation in vitro, thereby inducing cellular death (Mariussen et al., 2002).

The 3 mg/kg PCB dose did not result in any DPOAE deficits, whereas previous studies in our laboratory using the 3 mg/kg dose did find significant DPOAE deficits (Powers et al., 2006, 2009). This apparent discrepancy may be due to the fact that the rats in these previous studies were tested at an older age—approximately 200 days, whereas in this study, rats were tested at about 100 days of age. The rats in those studies had 100 more days of exposure to daily noise (e.g., room ventilation, laboratory and care staff personnel entering and exiting the room, and cage cleanings), and exposure to moderate noise over long periods can lead to damage of the outer hair cells and noise-induced hearing loss (Konings et al., 2009). Early exposure to this lower dose of PCBs may prime the outer hair cells to be more susceptible to the moderate noise experienced in everyday life, leading to a hearing loss that emerges as the animals age. We are currently conducting studies to determine whether early PCB exposure predisposes animals to noise-induced hearing loss later in life.

Although exposure to PBDEs alone at the doses we employed did not produce cochlear dysfunction, combined exposure to PBDE and PCB doses that alone did not have an effect on cochlear function did produce significant decreases in DPOAE amplitudes and increases in DPOAE thresholds across all frequencies tested. Coexposure to 3 mg/kg PCB plus 5.7 mg/kg PBDE produced effects roughly equivalent to the effect of a 6 mg/kg dose of PCBs alone, suggesting an additive effect on cochlear function. In contrast, exposure to 6 mg/kg PCBs plus 11.4 mg/kg PBDEs did not produce DPOAE deficits any greater than those observed with exposure to 6 mg/kg of PCBs alone, suggesting that this dose of the PCB mixture may produce a maximal effect on cochlear function. We have never tested higher doses of PCBs, so it remains unknown whether larger deficits in cochlear function could be produced with larger doses of PCBs. The lack of further deficits in the high combined group could be due to the fact that PCB exposure specifically targets the outer hair cells in the cochlea. Previous work has shown that developmental exposure to PCBs (Aroclor 1254) led to a loss of outer hair cells, but inner hair cells were not affected (Crofton et al., 2000a). Even severe damage that is specific to the outer hair cells may not cause more than a moderate hearing loss because the role of the outer hair cells is to act as a cochlear amplifier: increasing the sensitivity to low level sounds and fine tuning frequency discrimination. The inner hair cells and the auditory nerve can still be excited in the absence of the cochlear amplifier as measured by ABRs (Adelman et al., 2010).

Although the PCB and PBDE doses used in this study produce body burdens which are significantly higher than the levels found in most environmentally exposed humans, it is widely accepted according to the allometric scaling that higher administered doses are needed in rodents to produce the same internal doses and health effects observed in humans (Sarver et al., 1997; Sharma and McNeill, 2009). We estimate based on preliminary data that the internal PCB body burdens resulting from the doses we administered are similar to the body burdens of humans living near former PCB manufacturing sites in eastern Europe (Tmvec et al., 2008, 2010). Importantly, a recent study in Slovakia has confirmed that early exposure to PCBs was associated with decreased TEOAE and DPOAE amplitudes in 12-year-old children from one such contaminated site (Tmvec et al., 2010).

In summary, our data highlight that it is important not only to assess exposures to individual toxicants during critical periods of development but also to study mixtures of toxicants, as they may exist in the environment. Both the Fox River PCB mixture and DE-71 are environmentally relevant mixtures that individually pose health risks. Our findings contribute to the evidence that these structurally similar chemicals may have additive effects on various health endpoints, including hearing.
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